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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Cooper et al.
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THEREOF
Group: 1796
Examiner: Negrelli, Kara B.

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DECLARATION UNDER 37 CFR 1.132

Assistant Commissioner for Patents
Alexandria, VA 22313-1450

Sir:

I, Stephen Paul Rannard, hereby declare that:

1. I am a citizen of the United Kingdom.
2. My educational and technical background in the field of Chemistry is as follows:

(a) I received a B.Sc. (Hons) Degree in Chemistry and Materials Science from the University of Sussex in 1988.

(b) I received a D.Phil Degree in Polymer Science from the University of Sussex in 1994.

(c) I joined my present employer, the University of Liverpool, in 2007 and I currently have the title Professor located in Liverpool, United Kingdom.

3. I am a joint inventor of the invention described and claimed in Cooper et al., US Patent Application no. 10/587,732 filed May 17, 2007 and I have read Wu et al.

4. The following is my analysis in support of the above-cited Cooper et al. patent application.

5. **Detailed Comments**

Solubility

The art of Chemistry is complex but follows well understood rules. It is well known to persons skilled in the art that the solubility of cellulose acetate varies according to its degree of substitution of hydroxyl groups for acetyl groups. The water-soluble cellulose acetate of the current claims has different solubility parameters than the water-insoluble cellulose acetate of Wu et al..

For example, the PhD thesis of Collins Appaw at North Carolina State University entitled "Rheology and Microstructure of Cellulose Acetate in Mixed Solvent Systems" (attached hereto and available online at:

www.lib.ncsu.edu/theses/available/etd-01102005-140944/unrestricted/etd.pdf
) provides an excellent description of the background chemistry of cellulose acetate and its varying solubility, especially at page 2, lines 8-11 and 16-23; page 3, lines 4-6 and 12-21; page 10 (showing Figure 1); page 11 (showing Figure 2); page 14, lines 4-8, lines 13-14 and lines 20-23; page 15, line 17 to page 16, line 3; page 18, lines 12-16; and page 19, lines 10-20. This final paragraph in particular is most helpful in that it states:

The solution properties of cellulose acetates have been well studied and have been shown to be influenced by the average degree of substitution and the distribution of the substituents on the chain....cellulose acetates which have a degree of substitution (DS) of between 0.5 to 1 are soluble in water. This phenomena is attributed to disruption of the intra- and intermolecular hydrogen bonding within the cellulose system upon the introduction of the small number of acetyl groups. With increasing DS, cellulose acetates become insoluble in water but show good solubility in a variety of organic solvents such as THF, acetone....with cellulose acetates having high DS being insoluble in aqueous solution.

Also, reference to the attached USP4983730, in particular column 1, line 57 to column 2, line 5, confirms that "the term water soluble cellulose acetate is understood by persons skilled in the art to refer to cellulose acetate which dissolves in water relatively quickly and without leaving substantial amounts of insoluble residues" and that "whether a particular kind of cellulose acetate is water soluble will be readily apparent to those in the art".

In particular, although the terms "water-soluble" and "water-insoluble" are not standardized, but the skilled person would readily accept and understand the ranges defined as follows:

- "water-insoluble" means that the solubility of a material in water is less than 10 g/L at ambient temperature (20 °C); and conversely
- "water-soluble" means that the solubility of a material in water is greater than 10 g/L at ambient temperature (20 °C).

Emulsion Templating:

Removal of a solvent *prior* to freeze-drying results in a product which is not "emulsion-templated".

The phrase "emulsion-templating" can be explained, firstly, with reference to a webpage from the University of Liverpool: www.liv.ac.uk/chemistry/res/coopergroup/research/emulstemplate.html
Note that the webpage is for the Cooper Group (Andrew Cooper being one of the inventors of the present invention).

Secondly, reference to Figure 3 on page 509 of the article "Formation and Enhanced Biocidal Activity of Water-Dispersible Organic Nanoparticles" by H. Zhang *et al.* in Nature Nanotechnology, Vol. 3, August 2008, clearly shows what happens to the various components of an emulsion which is freeze-dried to form a "templated" porous body (i.e. having two types of pores: one from the sublimation of solid ice and one from the sublimation of the oil phase).

Finally, with specific reference to the question posed (how removal of a solvent *prior* to freeze-drying results in a product which is not "emulsion-templated"), enclosed is a series of SEM images showing, along the x-axis a varying percentage of oil phase (from 0 % to 75 %), and, along the y-axis different image bases (surface at 50 μm magnification, internal at 50 μm magnification and internal at 20 μm magnification). When there is 0 % oil phase, the structure (both surface and internal) is clearly not emulsion-templated. The structure shown is identical to one that would be produced if the oil was removed prior to freeze-drying. When 20 % to 75 % oil phase is present, the formation of large pores resulting from the presence of oil during freeze-drying can readily be seen – this is the process of emulsion-templating.

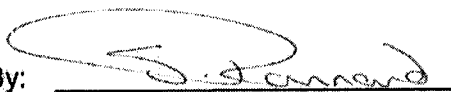
6. I conclude the following:

Solubility: The Cooper et al. patent application is reasonably directed to water soluble cellulose acetate which is substantially different from that of Wu et al.

Emulsion Templating: Emulsion templating differs from merely evaporating a solvent from a solution.

7. I declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and may jeopardize the validity of the application or any patent issuing thereon.

Dated: August 26th, 2010

By:  _____

Title: Professor of Chemistry
University of Liverpool, UK

ATTACHMENT 1

APPAW, COLLINS. Rheology and Microstructure of Cellulose Acetate in Mixed Solvent Systems (Under the direction of Dr. Saad A. Khan and Dr. John F. Kadla)

Cellulose is a natural abundant polymer used in a variety of applications. Its use however, is hampered by its poor solubility in various solvents. This is primarily due to the hydrogen bonds between the hydroxyl groups on the anhydroglucose chain. In view of that, various cellulose derivatives have been synthesized to aid dissolution and this impacts a variety of solubility characteristics. Cellulose acetates (CA) are cellulose esters that are partially substituted at the C-2, 3 and 6-positions of the anhydroglucopyranose residue. Their solubility in various solvents depends on the degree of substitution (DS) of the acetyl groups. For instance, CA is soluble in water at low DS of between 0.5-1. But it is insoluble in aqueous solutions at higher degree of substitution (DS > 1). CA is employed in various applications such as textile manufacture, tool handles, specialty papers, cigarette filters and is a polymer of choice in majority of reverse osmosis membrane preparation. These applications often exploit semi- to concentrated cellulose acetate solutions in appropriate solvents. Such systems can be induced to form aggregated structures such as gels which can be initiated by the addition of a non-solvent. Thus, depending on the solvent and non-solvent adopted, the cellulose acetate mixed solvent system can be tailored to exhibit sol-gel characteristics utilizing the inherent intra- and intermolecular interactions present in solution. However, such systems and the interactions influencing their behavior is not very well understood. In this regard, the main objectives of our study are as follows – to develop a

ternary mixed solvent system comprising of cellulose acetate, *N,N* dimethylacetamide and water and manipulate the system to form aggregated structures leading to phase separated gel network. The tools employed in this project to investigate and characterized the macroscopic properties as well as the microstructural changes are rheology, scanning electron microscopy (SEM) and laser scanning confocal microscopy (LSCM).

The first part of this study involves addition of water- *N,N* dimethylacetamide solutions in different ratios to bulk 20% cellulose acetate in *N,N* dimethylacetamide solutions with emphasis on increasing water content in the system. Using rheology as the main analytical tool, the steady state viscosity was found to increase with water content increase. Above water concentrations of 19%, there is a solution to gel transition, which also showed enhancement in dynamic viscoelastic properties with water content increment. The SEM micrographs showed similar patterns with gels having lower water content exhibiting larger voids in comparison to gels with higher water content at same cellulose acetate concentration. Using LSCM, we obtained microstructural formation with more open networks at lower water content, while a more compact homogenous structure was exhibited for higher water content gel samples.

In the second part of this study, addition of cellulose acetate to different ratios of *N,N* dimethylacetamide/water solutions are investigated. At low water content, the system showed steady state viscosity increase with water content as was observed in the first part of this thesis. Typically beyond 19% water concentration, the systems phase

separates into two layers consisting of a clear solution on top of a viscous bottom layer. This is in contrast to the first part of the study where we observed a uniform rigid material. Heating the two-phase system to 100°C and cooling back to room temperature led to the formation of a one-phase physical gel matrix. With increasing water content, the elastic and viscous moduli of the gels increased at constant cellulose concentration.

Finally, we investigate the gel properties with emphasis on yield stress when mechanical stress is applied to the gels. In addition, the gel-sol transition for the gels are investigated by subjecting them to temperature variations.

**RHEOLOGY AND MICROSTRUCTURE OF CELLULOSE ACETATE IN
MIXED SOLVENT SYSTEMS**

by
COLLINS APPAW

A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the requirements for the Degree of
Doctor of Philosophy

December, 2004

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Personal Biography

Collins Appaw was born in the city of Accra, which is located in Ghana, West Africa. His desire in pursuing a career in Chemical Engineering is primarily due to his interest in math and chemistry. His interest in research led him to pursue a Master program in Chemical Engineering at North Carolina A & T State University after completing his Bachelor of Science degree from the Department of Chemical Engineering, University of Science and Technology (UST) Ghana. After completing his Master degree, he immediately joined North Carolina State University to begin work on his doctoral research in the Fall of 2000. He plans to work in an industrial setting beginning 2005.

Acknowledgements

I will first of all to express my deepest gratitude to all whose help in various ways enabled me to complete this manuscript. First, I am grateful to my advisors Dr. Saad Khan and Dr. John Kadla, for their guidance and mentorship during the entire period I have been working on my research. I would like to thank Dr. Richard Gilbert and Dr. Orlin Velev for their contributions to my project from their fields of expertise and also for taking the time to serve on my committee. In addition, I would like to thank Dr. Spontak for his assistance in confocal microscopic analysis. I would also like to acknowledge the United States Department of Agriculture for funding this research study.

I enjoyed working and sharing some wonderful relationships with past and present members of the Rheology Group, Dr. Fedkiw's post-doc members and some members of Wood and Paper Science Department (Now at University of British Columbia, Canada), including Matthew Burke, Adeola Ali, Jeremy Walls, Jeff Yerian, Ahmed Abdala, Ahmed Eissa, Angelica Sanchez, Lauriane Scanu, Shamsheer Mahammed, Sachin Tawlar, David Franskowski, Satioshi Kubo, Quizon Dai, and Cameron Morris. I am especially indebted to my family for their love, support and encouragement through all the years. Finally, and most importantly, I would like to say thank you to my God and personal savior Jesus Christ.

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CHAPTER 1

INTRODUCTION AND OVERVIEW

Abstract

In this chapter, we offer the reader a brief introduction to the characteristics of cellulose and cellulose derivatives (cellulosics), in particular to cellulose acetate, the cellulosic studied in this dissertation. The natural abundance and biodegradability of cellulose together with its ability to provide unique (solution and end-use) properties through derivatization have made cellulosic polymers attractive for usage in a wide range of applications including textile fibers, molding powder sheets, optical membranes among others. This thesis involves the development and characterization of a ternary system consisting of a cellulose acetate, a solvent (*N,N* dimethylacetamide) and a non-solvent (water). We examine the rheology and microstructure of the system as a function of composition and processing parameters such as temperature. A variety of experimental techniques are employed to gain a fundamental understanding of how the mode of interactions impact cellulose acetate in the mixed solvent systems.

1.1 Introduction

Polymers and biopolymers are used in numerous applications with the most abundant biopolymer being cellulose.¹ We see an emergence in polymeric materials replacing traditional materials (e.g. metals) in many areas of applications in everyday products. This range from car body parts to everyday items such as cooking utensils. 5 These polymers are natural or synthetic products, which science and research have studied to investigate their applicability in various applications.

Cellulose is the most abundant natural polymer (Figure 1) that obtained its name from Payen in 1842.² The cellulose structure shows a chain-like extended linear macromolecule of anhydro-D-glucopyranose units linked at the 1 and 4 positions by glycosidic bonds.² The use of cellulose together with its derivatives has wide spread applications including fibers, films, plastics, coatings, suspension agents, composites.³ With the advent of synthetic polymer their use has somewhat dwindled, but some applications still adopt cellulose derivatives as the raw materials of choice. In addition, various studies are still been conducted to look for and expand their usage in existing 15 and new technologies. The inherent problem that faces users of cellulose is the general insoluble nature in most common solvents.⁴ There is therefore the need to modify the structure of cellulose to improve solubility which has led to the synthesis of various cellulose derivatives.⁵

Cellulose derivatives (cellulosics) comes in all forms and structures depending 20 on the functional group(s) used to substitute the hydroxyl groups on the cellulose chain.⁶⁻¹⁰ The derivatized cellulose molecule can be partially or fully substituted and this can go a long way in influencing solution and end-use behavior. To understand the

behavior of cellulose calls obviously for intensive research efforts in various disciplines. Knowledge of molecular level interactions will aid researchers to manipulate their properties and impart specific properties to the cellulosic system. Aqueous soluble cellulose such as methylcellulose are used as food-thickening agents
5 etc.³ Other classes of cellulose including cellulose acetate and cellulose nitrate are
used as cigarette filters and adhesives respectively. In spite of its potential for numerous applications though, both cellulose and its derivatives are being relegated to the background in preference to other polymeric materials. Thus, it is imperative to tap into and exploit the potential for using cellulose in existing technologies and develop new
10 applications for putting them to use. In view of that, we choose cellulose acetate as the primary polymer to study.

Cellulose acetate is a derivative of cellulose, which exhibits different solubility pattern depending on the degree of substitution of the hydroxyl units, which are replaced with O-acetyl groups. A typical cellulose acetate structure is shown in Figure
15 2 which has three acetyl groups on the chain and such units are termed as cellulose triacetate. The presence of acetyl and hydroxyl groups on the chain causes cellulose acetate to show interesting properties in various solvents.¹⁰ Therefore, the dissolution of cellulose acetate can be influenced depending on the affinity of a solvent for a specific functional group. For example, the rheology of cellulose acetate in different solvents is
20 postulated to be affected by specific interactions such as hydrogen bonding in solution.¹² The use of cellulose acetate usually involves concentrated solutions but unfortunately research in this area is few and far between.¹⁰ A possible explanation may be due to the viscous nature of relatively high cellulose acetate solutions making system

characterization difficult since most techniques such as traditional dynamic light scattering work best in dilute solutions.¹³ Therefore, to gain a concise understanding, which may lead to better predictions of cellulose acetate under such conditions, it is imperative to study semi- to high concentration levels. Thus, our ternary system comprising of cellulose acetate, *N,N* dimethylacetamide and water is studied at fairly high cellulose acetate concentrations. Phase separation and sol-gel transition is an important feature of our work since these characteristics are of considerable interest for application purposes. An example in this regard is the pharmaceutical industry, which is interested in phase separation characteristics in microencapsulation.¹⁴ We employ rheological techniques as our primary tool to investigate the macroscopic properties of our system. It must be emphasized that, rheology can also be used to predict the microstructural properties of various systems. Rheology has also been determined as the most direct and reliable method to determine sol-gel transition.¹⁵ Quite a number of cellulose gel and membrane system use scanning electron microscopy (SEM) to investigate microstructural changes. In view of this we adopted both SEM and a relatively new technique called laser scanning confocal microscopy (LSCM) to complement our rheological analysis. Our hope is the use of these techniques enabled us gain a fundamental understanding of how the mode of interactions impact cellulose acetate in mixed solvent systems.

1.2 Goals of Project

The main objective for this thesis is to examine the structure and property relationship between cellulose acetate and mixed solvents comprising *N,N* dimethylacetamide and water. The role of how polymer-cosolvent composition and processing parameters (e.g. temperature, cosolvent mixture) have on polymer solution properties inducing sol-gel transition in the system is examined. Furthermore, how such changes can affect the polymer conformation and interaction leading to aggregated structures (see Figure 3), and eventual phase-separation.

Several critical questions and issues need to be addressed in this project. They include:

- *How does polymer solvent interactions affect solution rheology and microstructure?*
- *Under what conditions can cellulose acetate form aggregation leading to phase-separated gels?*
- *How does process parameters (e.g. temperature) and sample preparation method influence rheology and microstructure?*

The specific step-by-step goals are as followings:

1. Develop a ternary system comprising of cellulose acetate, solvent and non-solvent

The presence of hydroxyl and acetyl groups in partially substituted cellulose acetate molecule enable the polymer architecture to be influenced by cosolvents with different solubility character. This prompted us to adopt two solvents, which are miscible with each other but having different solubility behavior for cellulose acetate. *N, N* dimethylacetamide is a good solvent for cellulose acetate and was chosen based on the

fact that, it can dissolve cellulose acetate to appreciable high concentrations. Also, other considerations included, high boiling point, low toxicity levels. Water the non-solvent for this study was chosen primarily because of its strong hydrogen bonding characteristics and obvious wide spread availability.

2. Examine the influence of cosolvent and cellulose acetate concentration pertaining to phase separation and sol-gel transition

The influence of non-solvent and how they can cause solutions of cellulose acetate to phase separate and eventually form elastic gel-like materials has been an important focus of this study. Phase separation in cellulose acetate system typically occurs when the solvent quality deteriorates. In this regard, we studied the effect of changing solvent quality by using different ratios of the cosolvents and progressively increase water content in the system. This process was also important to decipher the modes of hydrogen bond associations and dissociations in the system.

1.3 Overview of Thesis

In chapter 2, we provide a review of cellulose and cellulose derivatives. The chapter attempts to provide an investigative study of the various mechanism and mode of interactions exhibited by cellulosic systems. Details of the experimental methods adopted in our study are presented in chapter 3. Rheological methods are introduced and the basic concepts pertaining to the use of scanning electron microscopy (SEM) and laser scanning confocal microscopy (LSCM) to investigate the microstructure of the gel system are briefly outlined.

Chapter 4 deals with the rheological investigation of solutions and sol-gel mechanism of cellulose acetate, *N,N* dimethylacetamide solutions by the addition of different water concentrations (dissolution method 1). SEM studies are also performed to characterize and explain the gel system as a result of water content changes in the gels. In chapter 5, we examine a different route for solubilizing cellulose acetate in the cosolvents, which involves the addition of cellulose acetate to *N,N* dimethylacetamide/water solutions (dissolution method 2). This is in contrast to the solutions prepared in chapter 4 where we added water to cellulose acetate/ *N,N* dimethylacetamide solutions. Rheology and SEM were used to investigate the solutions and phase separated two-phase system formed by this alternative addition process. In chapter 6, we study the behavior of gels formed from both dissolution methods with respect to their response to stress and temperature changes. In addition we depict the microstructural properties of the gels using LSCM. Finally, we conclude the thesis with a summary of our findings and recommendations for future work in chapter 7.

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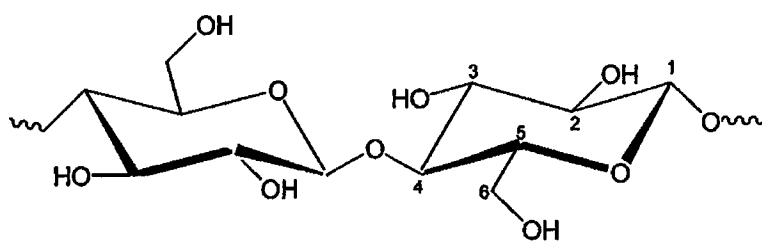


Figure 1. Chemical structure of cellulose.

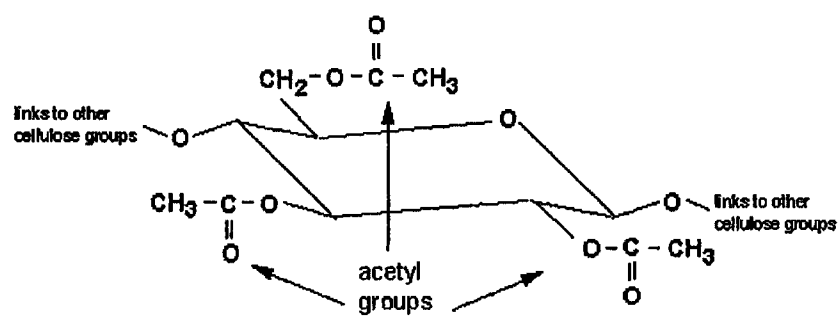


Figure 2. Schematic representation of a fully derivatized cellulose acetate chain.

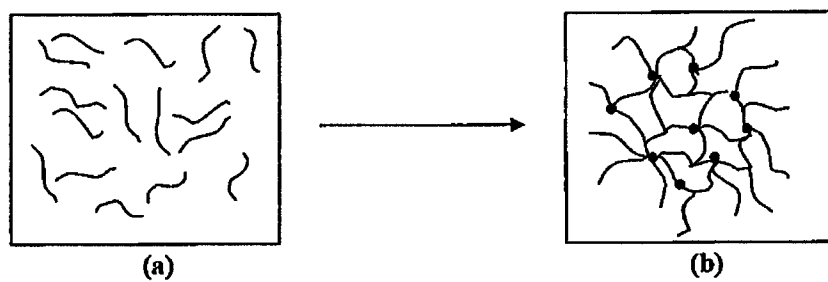


Figure 3. (a) Polymer in solution and (b) Aggregated network structure

CHAPTER 2

BACKGROUND AND LITERATURE REVIEW

Abstract

Cellulose is the principal constituent of higher plants (40-50%) and is also the main component of various kinds of natural fibers. In as much as cellulose has many commercial uses, cellulose is hindered by the inability to be dissolved by most solvents. There is therefore, the need to modify the cellulose structure by derivatization methods to partially or fully replace the hydroxyl groups on the cellulose chain with various other functional groups. This process yields cellulose derivatives, which exhibit better solubility characteristics and thus provide a wider range of usage. In this regard, we present a summary of cellulose and its derivatives and how their solution patterns are affected by different solvents. We then briefly discuss the factors contributing to gel formation in some cellulose derivatives and how the microstructure is affected by gelation. After which we shift the discussions to cellulose acetate, the cellulosic being investigated in this thesis with respect to solution and gelation characteristics in a variety of solvents.

2.1 Introduction to Cellulose and Cellulosics

Cellulose is the most abundant naturally occurring biopolymer.^{1,2} Various natural fibers such as cotton and higher plants have cellulose as their main constituent.^{2,3,4} Cellulose consists of long chains of anhydro-D-glucopyranose units (AGU) with each cellulose molecule having three hydroxyl groups per AGU, with the exception of the terminal ends.² Cellulose is insoluble in water² and most common solvents. The poor solubility is attributed primarily to the strong intramolecular and intermolecular hydrogen bonding between the individual chains.¹ In spite of its poor solubility characteristics, cellulose is used in a wide range of applications including composites, netting, upholstery, coatings, packing, paper, etc. Chemical modification of cellulose is performed to improve processability and to produce cellulose derivatives (cellulosics) which can be tailored for specific industrial applications as well to enable cellulosics to be characterized in the laboratory.⁵ Cellulose derivitization typically involves esterification or etherification of the hydroxyl groups on the cellulose chain.⁵ Cellulosics are in general strong, reproducible, recyclable and biocompatible,⁵ being used in various biomedical applications such as blood purification membranes and the like. Thus, through derivatization, cellulosics have opened a window of opportunity and have broadened the use of cellulosics. (Cellulosics have been around for ~100years! And actually used more as “plastics” before petroleum plastics came around)

Cellulose esters constitute the largest sector of commercially important cellulose derivatives. Of these, both organic esters such as cellulose acetates and cellulose acetate butyrates as well as inorganic esters such as cellulose nitrate and cellulose phosphate are widely produced. Together with cellulose xanthates, the cellulosic intermediate in

Rayon production, cellulose esters comprise more than 90% of the production capacity in the chemical processing of cellulose. The manufacturing of cellulose esters typically involves the reaction of cellulose with the corresponding organic / inorganic acid and a strong acid catalyst and depending on the substituent and degree of substitution are used
5 as textile fibers, coatings, cigarette filters, membranes, celluloid etc.³

By contrast, cellulose ethers are manufactured through alkali reactions,⁵ are typically water soluble and widely used to modify the rheological properties in industrial applications such as paint, oil recovery, food and cosmetics.⁶ Some of the most common cellulose ethers are methyl cellulose, hydroxyethyl cellulose and
10 carboxymethyl cellulose (CMC), with CMC dominating by far.

The utilization of cellulose and cellulose derivatives require a comprehensive understanding of the physical and chemical properties and behavior of the various derivatives. In view of that, more research is needed to better understand at the molecular level the various interactions and mechanisms influencing their properties
15 and behavior.

Solution characteristics of cellulose derivatives

Cellulose derivatives exhibit different solution properties depending on the solvent system and the functional group(s) used to substitute the hydroxyl group(s) on cellulose chain. Cellulose esters such as cellulose nitrate and cellulose acetate dissolve in a wide range of solvents.²⁰ For instance, cellulose acetates exhibit solubility characteristics in both aqueous and common organic solvents, such as chloroform (CH₂Cl₂), acetone and DMF depending on the DS. Likewise, cellulose ethers such as

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methyl cellulose and other hydrophobically modified cellulose derivatives are soluble in aqueous solutions as well as some organic solvents depending on the degree of substitution (DS) and preparation method.⁷⁻¹⁶ Similarly, factors such as temperature, concentration, shear rate and degree of substitution have been found to affect the molecular structure of hydroxypropyl cellulose (HPC) solutions.¹⁷ HPC gels have been shown to exhibit **5** greater elasticity with increased propylene glycol (PG) content in mixtures of water and ethanol.¹⁸ It has been observed that for aqueous carboxymethyl cellulose (CMC) solutions factors such as molar mass and concentration of the dissolved CMC are the major factors affecting its rheological properties.¹⁹ In low CMC concentration solutions Newtonian behavior was observed, while pseudoplastic, thixotropic and viscoelastic behavior was exhibited at high concentrations.²⁰ It has been shown that in sodium CMC solutions crystalline regions act as binding or crosslinking sites and depending on the solvent or salt type lead to the formation of swollen gels or aggregates in solution.²¹

Gelation of cellulose ethers and esters

The inception of gelation is initiated by large macromolecular associations forming clusters, which lead to an infinite homogenous cluster extending through entire volume of the system.²² Solution to gel transitions have been observed in various cellulose derivatives, forming mostly physical gel systems. It is widely accepted that a physical polymer gels is initiated by physical aggregation leading to the formation of a three-dimensional network.²² The transitions from solutions to gels have been studied using various techniques including rheological methods.^{5, 7-8} Rheology has been

determined to be the most direct method to determine sol-gel transitions in polymeric systems.¹² Not only can rheology be used to investigate the gelation mechanism, it also provides important characterization properties which invariably assist in assigning specific applications for polymers. This technique has been applied to various cellulosic systems to precisely determine and control gel formation. Phase separation behavior is often exhibited by cellulosic systems.²³⁻²⁴ It has been shown that phase separation and gelation are two characteristics which are closely related.²⁵ Gelation in cellulosic systems typically exhibits phase separation characteristics prior to gelation. Phase separation in cellulosic solvent systems, are dependent on the concentrations of specific derivative and solvents used.²⁶⁻²⁷ This phenomena typically occurs when solvent quality deteriorates or changes in the polymer-solvent interactions occur due to external factors such as temperature, non-solvent addition etc.²⁸⁻²⁹ These rearrangement of the intra- and intermolecular interactions in solution due the above-mentioned factors may account for gel formation in such cellulose systems. A variety of water-soluble cellulose exhibit a unique thermoreversible gelation process.⁵⁻¹⁰ The unique feature of these systems is the induction of a three-dimensional network and gel formation with increasing temperature, then the return to an isotropic solution when cooled.⁶ Such thermoreversible behavior is observed for methyl cellulose solutions and has been attributed by some researchers to hydrophobic interactions in solution. Manipulation of methyl cellulose gelation has been accomplished through the addition of salting-in and salting-out salts.³⁰⁻³¹ Sodium chloride, a typical salting-out salt has been shown to aid gelation,³⁰ while sodium iodide (salting-in salt) inhibited gel formation.³¹ Likewise, the

use of ionic surfactants have been shown to greatly affect gel network strength in other aqueous cellulose derivative systems.²⁵

Unlike cellulose ethers, reports involving gel formation of cellulose esters are rather limited and may be due in part to their general decline in such applications. Notwithstanding gelation has been observed in a variety of cellulose ester systems.³²⁻³³ Gels 5 prepared from cellulose esters usually find applications such as filters and binding agents.³⁴ Separation of metals in aqueous solutions have been performed using cellulose nitrate gel matrixes,³⁴ while cellulose phosphate gels have been synthesized to be used in orthopedic applications especially for bone regeneration.³⁵

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2.2 Physical, Chemical and Solution Properties of Cellulose Acetate

Cellulose acetates (CA) are one of the most widely produced cellulose esters. It is an acetylated cellulosic in which the hydroxyl groups along the cellulose chain are fully or partially acetylated in a statistical manner.³⁶⁻⁴² All of the industrial processes practiced today are aimed at the manufacture of a fully substituted cellulose triacetate 15 (DS> 2.9, or an acetyl content of 44.8%) as the primary product.⁴² This is either isolated and processed as is or partially desubstituted to "secondary acetate" with a DS of between 1.8 and 2.5 (predominately near 2.4).⁴² A heterogeneous process to produce a uniform diacetate is not currently available. The majority of CA is produced using a solution process, although fiber acetylation is also practiced. Typically cotton linters or 20 softwood sulfite or prehydrolyzed sulfite pulps are used as the raw material. In solution acetylation either glacial acetic acid alone or in combination with methylene chloride is employed as a solvent for the CTA formed, and the reaction is catalyzed using sulfuric

acid. In secondary acetate production, the CTA is not isolated rather being immediately converted to the diacetate by the addition of water, dilute acetic acid or NaOH. This process affectively decomposes the intermediate sulfate ester groups in the chain and decrease the DS while still maintaining the cellulose acetate in solution. The
5 manufacture of CA differs in the solvent used for the acetylation and process conditions adopted.⁴¹ Acetic acid process by far represents the route for producing the largest amount of cellulose acetate for commercial purposes. With development of new solvents for cellulose, new path for acetylation processes are still being investigated since acetylation require dissolution of cellulose.⁴²

10 The solution properties of cellulose acetates have been well studied and have been shown to be influenced by the average degree of substitution and the distribution of substituents along the chain.⁴⁰ The structural properties of CA affect the rheology of the cellulose acetate-solvent system. Cellulose acetates which have degree of substitution (DS) of between 0.5 to 1 are soluble in water.⁴³⁻⁴⁴ This phenomena is attributed to disruption of the intra- and intermolecular hydrogen bonding within the cellulose system upon the introduction of the small number of acetyl groups.⁴⁴ With increasing DS, cellulose acetates becomes insoluble in water but show good solubility in a variety of organic solvents, such as tetrahydrofuran (THF), acetone, *N,N* dimethylacetamide (DMAc) with cellulose acetates having high DS being insoluble in
15 aqueous solution. Similarly, depending on the DS, concentration and solvent cellulose acetates exhibit a variety of solution properties. Boerstael *et al.* found that over a DS range of 0.23 to 2.89, cellulose acetate was soluble in phosphoric acid and exhibited liquid crystalline properties.⁴⁵ Using dilute solutions of cellulose diacetate (CDA) in
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mixed solvents it has been reported that basic solvents (e.g. acetone) interact primarily with the hydroxyl groups on the cellulose chain, while acidic solvents (e.g. formic acid) primarily solvate the acetyl groups.⁴⁶ Therefore, the use of specific solvents can induce structural changes in solution depending on the amount of acetyl and hydroxyl groups on partially substituted CA chains. This in some cases lead to phase separation and/or gelation in a variety of CA solvent systems. Matsuyama *et al.*⁴⁷ used supercritical CO₂ to cause phase separation and was observed to occur instantaneously when CO₂ was added to cellulose acetate in acetone, methyl acetate, 1,3-dioxolane and 2-butanone solutions respectively. CA samples with different molecular weight and degree of substitution have been showed to form gels in dimethyl phthalate.⁴⁸ Increase in degree of substitution of CA and polymer concentration in the system, causes an increase in the dynamic moduli.⁴⁸

2.3 Applications and New Emerging Uses of Cellulose Acetate

The use of CA dates back to the early 1900s when the first photographic film was produced by the Dreyfus brothers.⁴⁹ Properties including good toughness, deep gloss and a “natural” feel have made CA attractive for a number of applications. It also has the advantage of possessing low toxicity. CA is used in a variety of industrial applications such as textile manufacture, tool handles, speciality papers, cigarette filters (major constituent in the U.S. for producing cigarette filter tow), among others. Factors such as transparency, smoothness and color have allowed CA to be used in handles for toothbrushes, umbrellas and screwdrivers. Spectacle frames have been made from sheets of cellulose acetate, but this practice has been replaced to some extent with other

synthetic thermoplastic materials. CA is increasing being utilized in the manufacturing of separation and filtration media in such applications as electrophoresis and reverse osmosis membranes (ROM).⁵⁰⁻⁵¹ As well, CA has shown good promise in the area of drug delivery and research is underway to determine its effectiveness and applicability.

2.4 Gelation Behavior of Cellulose Acetate

Previous work on the gelation mechanism of cellulose acetate has shown interesting behavior with respect to the sol-gel transition. CA gels exhibit thermal reversible properties, which depend on factors such as CA concentration, acetyl content and the type of solvent. Unlike other cellulose esters which gel when heated, CA solutions do not exhibit such gel characteristics. In most CA/solvent systems gelation occurs after the CA solution is heated to a specific temperature and subsequently cooled. Such behavior has been reported for CA/benzyl alcohol solutions.⁵²⁻⁵³ Heating benzyl alcohol solutions of CA to 60-80°C led to gelation when the system was cooled to 25°C. It is postulated that gel formation is induced by the existence of strong intermolecular associations in the system.⁵²

In polymeric systems physical gel formation can arise as a result of phase separation. Depending on the system phase separation leads to polymer aggregation and the formation of large macromolecular assemblies.²² In some CA/solvent systems the addition of a non-solvent (e.g. water) can induce gelation in which phase separation characteristics are exhibited prior to gel formation. Phase separation is typified by the observation of a gradual to extreme cloudiness in the system and is dependent on the

concentration of polymer and cosolvents used. Such phase separation induced CA gelation is used in the production of wet phase inversion membranes.⁵⁴ Here water is the nonsolvent of choice owing to its nontoxic nature, widespread availability and strong hydrogen-bonding characteristics. However, the application of other nonsolvents such as alcohol-water mixtures have been studied.^{55,56}

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ATTACHMENT 2

United States Patent [19]

Domeshek et al.

[11] Patent Number: 4,983,730

[45] Date of Patent: Jan. 8, 1991

[54] WATER SOLUBLE CELLULOSE ACETATE COMPOSITION HAVING IMPROVED PROCESSABILITY AND TENSILE PROPERTIES

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[73] Assignee: Hoechst Celanese Corporation, Somerville, N.J.

[21] Appl. No.: 240,875

[22] Filed: Sep. 2, 1988

[51] Int. Cl.³ C08B 3/06; A61K 9/30; A61K 9/36

[52] U.S. Cl. 536/69; 424/475; 424/480; 427/3; 106/196

[58] Field of Search 536/69; 424/475, 480; 427/3; 106/196

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Primary Examiner—Nathan M. Nutter
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[57] ABSTRACT

Water soluble cellulose acetate composition having improved processability and tensile properties suitable for application as films, coatings and fibers and comprising one or more lower molecular weight water soluble cellulose acetate components and one or more higher molecular weight water soluble cellulose acetate components, and wherein the lower molecular weight water soluble cellulose acetate components have solution viscosities at least 20 percent of that of the higher molecular weight water soluble cellulose acetate components. An exemplary composition comprises from about 85 to 98 percent by weight of a lower molecular weight water soluble cellulose acetate component having a solution viscosity of from about 5 to 50 cps and from about 15 to 2 percent by weight of a higher molecular weight water soluble cellulose acetate component having a solution viscosity of greater than 100 cps.

18 Claims, No Drawings

WATER SOLUBLE CELLULOSE ACETATE COMPOSITION HAVING IMPROVED PROCESSABILITY AND TENSILE PROPERTIES

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to a water soluble cellulose acetate composition for application as films, coatings and fibers. More particularly the present invention relates to a composition comprising a lower molecular weight water soluble cellulose acetate component and a higher molecular weight water soluble cellulose acetate component.

Water soluble cellulose acetate films and fibers and processes for producing them are known in the art. U.S. Pat. No. 2,129,052 to Fordyce, U.S. Pat. No. 2,448,082 to Creamer, U.S. Pat. No. 3,482,011 to Bohrer and U.K. Pat. No. 696,903 to Davoud all disclose that water soluble and water susceptible cellulose acetate film and fibers can be produced by utilizing various esterification and/or hydrolysis techniques on cellulose acetate which is normally insoluble in water. Additionally, Russian Pat. No. 1740744 discloses using water soluble cellulose acetate as a tablet binder for use by the pharmaceutical industry.

These water soluble cellulose acetate films and fibers, however, often have limited utility because they are difficult to process or have low tensile properties or both.

SUMMARY OF THE INVENTION

To this end, the present invention provides a water soluble cellulose acetate composition which has both improved processability and tensile properties. The water soluble cellulose acetate composition comprises one or more lower molecular weight water soluble cellulose acetate components and one or more higher molecular weight water soluble cellulose acetate components. The lower molecular weight water soluble cellulose acetate components typically have viscosities at least 20 percent and preferably at least 60 percent lower than that of the higher molecular weight water soluble cellulose acetate components. For example, the composition may comprise a lower molecular weight water soluble cellulose acetate component having a viscosity of from about 5 to 50 cps and a higher molecular weight water soluble cellulose acetate component having a viscosity of greater than 100 cps. These water soluble cellulose acetate compositions have applications as films and fibers and especially as coatings for tablets for use by the pharmaceutical industry.

DETAILED DESCRIPTION OF THE INVENTION

Cellulose acetate is obtained by esterifying cotton linters or wood pulp. The term water soluble cellulose acetate is understood by persons skilled in the art to refer to cellulose acetate which dissolves in water relatively quickly and without leaving substantial amounts of insoluble residues. Typically, water soluble cellulose acetate has a degree of substitution ranging from about 0.5 to 0.9 with 0.6 to 0.8 being preferred. It is to be recognized that "degree of substitution" is just one of several conventional ways that is used to describe the type of cellulose acetate which is water soluble. Other common ways to describe this type include measuring the acetyl value or acetyl content, measured as weight

percent acetyl or weight percent acetic acid. The particular method used to describe the cellulose acetate of the present invention is not critical, and whether a particular kind of cellulose acetate is water soluble will be readily apparent to those in the art. Also it is recognized that a cellulose acetate composition which is soluble in water will be soluble in other organic solvents such as formamide, N, N-dimethylformamide, dimethyl sulfoxide, pyridine and N-methyl-2-pyrrolidone, mixtures thereof and mixtures thereof with water.

The present invention provides an improved water soluble cellulose acetate composition wherein water soluble cellulose acetate components of different molecular weights are combined to form water soluble cellulose acetate blends which have improved processability and tensile properties as compared to unblended compositions of water soluble cellulose acetate. Typically, molecular weight is described in terms of solution viscosity, and water soluble cellulose acetate is marketed based on "grades" thereof depending on the solution viscosity. Using a capillary viscometer to measure the solution viscosity of a six percent solids solution, a low viscosity grade water soluble cellulose acetate has a solution viscosity of from about 5 to 50 cps, a medium/low viscosity grade water soluble cellulose acetate has a solution viscosity of from about 50 to 100 cps, a medium viscosity grade water soluble cellulose acetate has a solution viscosity of from about 100 to 250 cps and a high viscosity grade water soluble cellulose acetate has a solution viscosity of greater than about 250 cps.

As described in U.S. Pat. No. 2,129,052 to Fordyce and herein incorporated by reference, water soluble cellulose acetate is typically produced from cellulose acetate by dissolving it in acetic acid and water and thereafter continuing the deacetylation. The reaction temperature, sulfuric acid catalyst level and reaction time determine the molecular weight and resultant solution viscosity of the water soluble cellulose acetate. The water soluble cellulose acetate is typically in the form of a solid such as a powder or flake or in solution.

Water soluble cellulose acetate grades having a lower solution viscosity (low viscosity grade and medium/low viscosity grade) tend to flow easily in the liquid state and have the ability to dissolve higher amounts of solids, but in the solid state they tend to have poorer tensile properties as compared to other water soluble cellulose acetate grades having a higher viscosity. Medium viscosity grade water soluble cellulose acetate and high viscosity grade water soluble cellulose acetate are typically the opposite. They have more resistance to flow in the liquid state but have improved tensile properties in the solid state. By utilizing the present composition, a blend or mixture of grades of water soluble cellulose acetate may be formed and the processing benefits of the lower viscosity grade water soluble cellulose acetate and the tensile property benefits of the higher viscosity grade water soluble cellulose acetate are both realized. This is unexpected in that an unblended water soluble cellulose acetate composition having the same solution viscosity as the water soluble cellulose acetate composition formed from a blend of grades of water soluble cellulose acetate will have processability properties or tensile properties or both properties which are significantly poorer compared to the present blended composition. Moreover, when polymer compositions are formed from blends of similar polymer compounds having different molecular weights, an unusable prod-

uct is sometimes obtained in that the poorer properties or features of these compounds are retained or accentuated.

The present composition comprises one or more lower molecular weight water soluble cellulose acetate components and one or more higher molecular weight water soluble cellulose acetate components. The compositions are formed by blending and homogenizing them together employing the components in their powder, flake, solution or melt form using agitation, melt blending, solution blending and other conventional blending and homogenizing techniques.

Preferably the solution viscosities of the lower molecular weight water soluble cellulose acetate components are at least 20 percent lower than that of the higher molecular weight water soluble cellulose acetate component and preferably are at least 60 percent lower than that of the higher molecular weight water soluble cellulose acetate components. For example, a composition comprising from about 85 to 98 percent by weight of a lower molecular weight water soluble cellulose acetate component having a solution viscosity of from about 5 to 50 cps and from about 2 to 15 percent by weight of a higher molecular weight water soluble cellulose acetate component having a viscosity of greater than 100 cps will retain the processability characteristics of the low viscosity grade water soluble cellulose acetate component and will retain the tensile properties of the higher viscosity grade water soluble cellulose acetate component. It is to be recognized that the addition of relatively low amounts of the higher molecular weight component, such as from about 1-2 percent by weight, will have an appreciable affect on the tensile properties of the composition as compared to the unblended low viscosity grade water soluble cellulose acetate composition. In general, the compositions will have improved tensile properties as compared to an unblended low viscosity grade water soluble cellulose acetate composition and improved processability properties as compared to an unblended high viscosity grade water soluble cellulose acetate composition.

Plasticizers also may be added to any of the water soluble cellulose acetate components to modify the processability and tensile properties such as by lowering the glass transition temperature and the melting point of the composition. Exemplary plasticizers include glycerin, polyethylene glycols, diethylene glycols, propylene glycol and dimethyl sulfoxide. The plasticizers typically function to facilitate processing and to increase the flexibility and toughness of the final product.

In operation, the present water soluble cellulose acetate composition is obtained by first forming a blend of one or more of the lower molecular weight components and of one or more of the higher molecular weight components preferably in their solid forms. This blend is then formed into a homogeneous solution by dissolving the blend in water using agitation or stirring. The solution is then formed into an article of manufacture such as a coating, film or fiber.

If used as a coating, a preferred application is as tablet coatings for the pharmaceutical industry. The present water soluble cellulose acetate blend is dissolved in a solution and is sprayed or atomized through a small nozzle onto a tablet. The present water soluble cellulose acetate composition may dissolve higher amounts of solids while retaining its processability. This quality permits flow through the nozzle at higher throughput, increases production capacity and results in uniformly

coated tablets. Once coated, the coating must be durable and thus the improved tensile properties of the present composition are also important. Similarly, the improved processability and tensile properties facilitate the production of films using casting, melt extrusion and other film-making techniques and facilitate the production of fibers using extrusion or other fiber-making techniques.

The invention is additionally illustrated in connection with the following examples, which are to be considered as illustrative of the present invention. It should be understood, however, that the invention is not limited to the specific details of the examples.

EXAMPLE 1

90 percent by weight of a powdered, lower molecular weight water soluble cellulose acetate composition having a viscosity of 12 cps was blended using agitation with a 10 percent by weight powdered, higher molecular weight water soluble cellulose acetate composition having a viscosity of 147 cps. The powdered blend was put into solution by dissolving in water and the resistance to flow was checked visually. The solution was cast onto a sheet of glass and uniformly spread thereon. The solution was dried to form a film. The tensile strength and percent elongation of the film were measured using an Instron® Model 1122.

EXAMPLE 2

Following the procedures of Example 1, a film comprising 60 percent by weight of a lower molecular weight water soluble cellulose acetate component having a solution viscosity of 12 cps and 40 percent by weight of a higher molecular weight water soluble cellulose acetate component having a solution viscosity of 147 cps was produced and the resistance to flow and tensile properties thereof determined.

EXAMPLE 3

Following the procedures of Example 1, a film comprising 45 percent by weight of a lower molecular weight water soluble cellulose acetate component having a solution viscosity of 12 cps and 55 percent by weight of a higher molecular weight water soluble cellulose acetate component having a solution viscosity of 147 cps was produced and the resistance to flow and tensile properties thereof determined.

EXAMPLE b 4-5

For comparison purposes, following the procedures of Example 1, films comprising 100 percent low viscosity grade water soluble cellulose acetate having a solution viscosity of 12 cps (Example 4) and a composition comprising 100 percent medium viscosity grade water soluble cellulose acetate having a solution viscosity of 147 cps (Example 5) were produced and the resistance to flow and tensile properties thereof determined.

Table 1 illustrates the resistance to flow and tensile properties of the compositions of Examples 1-5 and includes measurements for specific tensile properties, namely the tensile strength and the percent elongation.

TABLE 1

Example No.	Resistance to Flow	Tensile Strength (psi)	% Elongation
1	Low	7185	3.79

TABLE 1-continued

Example No.	Resistance to Flow	Tensile Strength (psi)	% Elongation
2	Low	7163	7.63
3	Low	7522	10.02
4	Low	3261	1.93
5	High	7299	7.65

As is readily apparent, a water soluble cellulose acetate composition produced according to Examples 1-3 results in a product which has the processability characteristics of a lower viscosity water soluble cellulose acetate, namely its resistance to flow is low, and has the higher tensile properties of a higher viscosity water soluble cellulose acetate. The unblended low viscosity grade water soluble cellulose acetate (Example 4) on the other hand has poor tensile properties and the high viscosity grade water soluble cellulose acetate (Example 5) is resistant to flow.

The principles, preferred embodiments and modes of operation of the present invention have been described in the foregoing specification. The invention which is intended to be protected herein, however, is not to be construed as limited to the particular forms disclosed since those are to be regarded as illustrative rather than restrictive. Variations and changes may be made by those skilled in the art without departing from the spirit of the invention.

That which is claimed is:

1. A water soluble cellulose acetate composition having improved processability and tensile properties suitable for application as films, coatings and fibers and comprising one or more lower molecular weight water soluble cellulose acetate components and one or more higher molecular weight water soluble cellulose acetate components, and wherein said lower molecular weight water soluble cellulose acetate components have solution viscosities at least 20 percent lower than that of said higher molecular weight water soluble cellulose acetate components.

2. A water soluble cellulose acetate composition according to claim 1 additionally including a plasticizer.

3. A water soluble cellulose acetate composition according to claim 2 wherein said plasticizer is selected from the group consisting of glycerin, polyethylene glycols, diethylene glycols, propylene glycol and dimethyl sulfoxide.

4. A water soluble cellulose acetate composition according to claim 1 wherein said higher molecular weight component is present in an amount greater than about 1 percent by weight.

5. A water soluble cellulose acetate composition according to claim 1 wherein said lower molecular water

soluble cellulose acetate components have solution viscosities of at least 60 percent lower than that of said higher molecular weight water soluble cellulose components.

6. A coating comprising the water soluble cellulose acetate composition of claim 1.

7. A pharmaceutical tablet coated with the coating composition of claim 6.

8. A water soluble cellulose acetate composition having improved processability and tensile properties suitable for applications as films, coatings and fibers and comprising a low molecular weight water soluble cellulose acetate component having a solution viscosity of from about 5 to 50 cps and a higher molecular weight water soluble cellulose acetate component having a solution viscosity of greater than about 100 cps.

9. A water soluble cellulose acetate composition according to claim 8 additionally including a plasticizer.

10. A water soluble cellulose acetate composition according to claim 9 wherein said plasticizer is selected from the group consisting of glycerin, polyethylene glycols, diethylene glycols, propylene glycol and dimethyl sulfoxide.

11. A water soluble cellulose acetate composition according to claim 8 wherein said higher molecular weight component is present in an amount greater than about 1 percent by weight.

12. A coating comprising the water soluble cellulose acetate composition of claim 8.

13. A pharmaceutical tablet coated with the coating composition of claim 12.

14. A water soluble cellulose acetate powdered blend having improved processability and tensile properties comprising from about 85 to 98 percent by weight of a low molecular weight water soluble cellulose acetate component having a solution viscosity of from about 5 to 50 cps and from about 15 to 2 percent by weight of a higher molecular weight water soluble cellulose acetate component having a solution viscosity of greater than 100 cps.

15. A water soluble cellulose acetate composition according to claim 14 additionally including a plasticizer.

16. A water soluble cellulose acetate composition according to claim 15 wherein said plasticizer is selected from the group consisting of glycerin, polyethylene glycols, diethylene glycols, propylene glycol and dimethyl sulfoxide.

17. A coating comprising the water soluble cellulose acetate composition of claim 14.

18. A pharmaceutical tablet coated with the coating composition of claim 17.

* * * * *

ATTACHMENT 3



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Emulsion Templating

Emulsions are heterogeneous mixtures of at least one miscible liquid dispersed in another in the form of droplets. In most cases, at least one of the liquids will be water or an aqueous solution. An emulsion is often described as either oil-in-water (O/W) or water-in-oil (W/O) where the first phase mentioned refers to the internal (or dispersed) phase. In the context of polymer synthesis, emulsions can be used in three ways (Fig. 1) as "templates" for the preparation of colloids, porous materials, and composite materials, respectively. Emulsion templating is a flexible and easily-controlled method for the fabrication of macroporous materials (pore size range 5–100 μm) by polymerising the continuous phase of a high internal phase emulsion (HIPE) (internal phase volume > 74.05 %). If a less concentrated emulsion is used (internal phase volume < 60 %), a more closed-cell porous structure will be obtained.

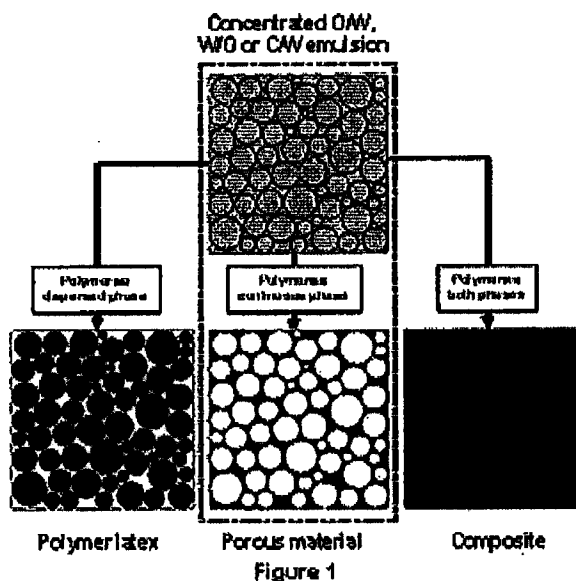


Figure 1

In our research, high internal phase oil-in-water (O/W) emulsions are used to prepare hydrophilic polymeric materials. It is then extended to make hierarchically porous inorganic materials (such as silica, alumina, zirconia, titania), metals (such as gold, palladium), and site-isolated nanocomposites (such as gold nanoparticles in silica). To avoid the use of a large amount of organic solvents in the emulsion, compressed CO_2 has been used to produce the emulsions and porous structures. We have also developed hydrocarbon surfactants to stabilize CO_2 -in-water emulsions with the volume ratio of CO_2 phase up to 90 %. As a result of these stable CO_2 emulsions, highly porous polyacrylamide and poly(vinyl alcohol) have

been prepared. Below gives a few images of emulsion-templated porous materials.

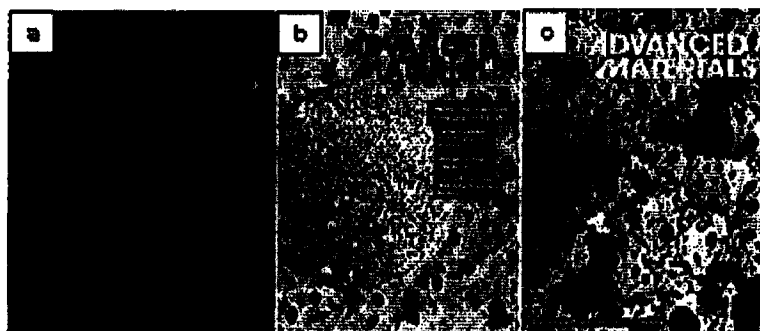


Figure 2: (a) Porous silica structure, scale bar 20 μm ; (b) Emulsion-templated gold beads; (c) Porous polyacrylamide made from CO_2 -in-Water emulsion

ATTACHMENT 4

Formation and enhanced biocidal activity of water-dispersible organic nanoparticles

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Water-insoluble organic compounds are often used in aqueous environments in various pharmaceutical and consumer products. To overcome insolubility, the particles are dispersed in a medium during product formation¹, but large particles that are formed may affect product performance and safety. Many techniques have been used to produce nanodispersions—dispersions with nanometre-scale dimensions—that have properties similar to solutions^{2–4}. However, making nanodispersions requires complex processing, and it is difficult to achieve stability over long periods¹. Here we report a generic method for producing organic nanoparticles with a combination of modified emulsion-templating⁵ and freeze-drying. The dry powder composites formed using this method are highly porous, stable and form nanodispersions upon simple addition of water. Aqueous nanodispersions of Triclosan (a commercial antimicrobial agent) produced with this approach show greater activity than organic/aqueous solutions of Triclosan.

As the range of nanoparticle-based technologies increases, issues of sustainability and environmental impact become more important. Standard guidelines for evaluating long-term chemical risks/benefits, such as environmental persistence, bioaccumulation and nanoparticle toxicity have been suggested recently⁶. In general, insoluble inorganic nanoparticles are expected to be more environmentally persistent than organic nanomaterials, because even poorly soluble organic compounds are ultimately completely soluble in water.

Drug solubility controls circulating plasma levels, pharmacokinetics and bioavailability of orally-dosed particulate pharmaceuticals that are poorly water soluble⁷. Although medical applications have received considerable attention, significant opportunities still remain for nanodispersions of other commercial low-solubility organic molecules, especially as their environmental fate is well understood.

Aqueous nanodispersions offer the benefits of dispersing poorly water-soluble molecules without directly using organic solvents. Although many organic nanoparticle production methods exist^{3,4}, few are truly generic approaches. For example, wet-milling is time- and energy-intensive, transforming bulk organic materials into nanoparticles³, and is incompatible with explosive, low-melting, or temperature-sensitive compounds. Moreover, 'milling agent' degradation introduces impurities, and

producing uniform organic nanoparticles with diameters <300 nm is difficult owing to particle aggregation⁷.

Horn and Rieger⁴ comprehensively reviewed the main classes of solvent-based nanoparticle formation routes, described mainly as 'precipitation and condensation processes'. Our new route to aqueous nanodispersions differs from these classifications and other techniques including salting-out⁸, film rehydration^{9,10} and vesicle formation¹¹. We use steps used previously to produce so-called 'emulsion-templated'¹² or 'polyHIPE' polymers⁵ without using chemical crosslinking to maintain the porous structure. Initially, we generate an oil-in-water (O/W) emulsion using a volatile organic solvent oil phase containing a dissolved organic compound, and a continuous aqueous phase containing a stabilizer or mixture of stabilizers (for example, water-soluble polymers or surfactants). The emulsion is dripped or atomized onto the surface of a cryogenic liquid, or directly frozen, resulting in the formation of frozen beads (2–3 mm diameter), micrometre-sized powders or large monolithic structures. Finally, both the water and the organic solvent are removed by freeze-drying, generating dry composite materials comprising the water-insoluble organic compound and water-soluble polymers/surfactants. The highly porous composites dissolve readily in water, releasing the organic compound as nanoparticulate dispersions, which resemble transparent molecular solutions (Fig. 1a–c).

The process was exemplified using a model water-insoluble compound, Oil Red (OR) dissolved in cyclohexane¹³ (CH, 0.018 wt%), and poly(vinyl alcohol) (PVA) and sodium dodecyl sulphate (SDS) dissolved in water (O/W ratio = 75:25; 5 wt% PVA, 5 wt% SDS). The porous emulsion-templated structures (Fig. 1d–e) and spheroidal OR nanoparticles (Fig. 1f) were revealed by scanning electron microscopy (SEM) and scanning transmission electron microscopy (STEM) (Fig. 1g). The materials dissolve rapidly in water, producing clear red OR nanodispersions, visually identical to solutions (see Supplementary Information, Movie S1). Analysis of the nanodispersions by dynamic light scattering (DLS) showed an average particle diameter of ~90 nm (Fig. 1h) and smaller aggregates (see Supplementary Information, Fig. S1) ascribed to micelles or PVA/SDS complexes¹⁴.

As OR has a low CH solubility (<0.35 wt%), acetone co-solvent was added at low levels (0.5 wt%) allowing dye

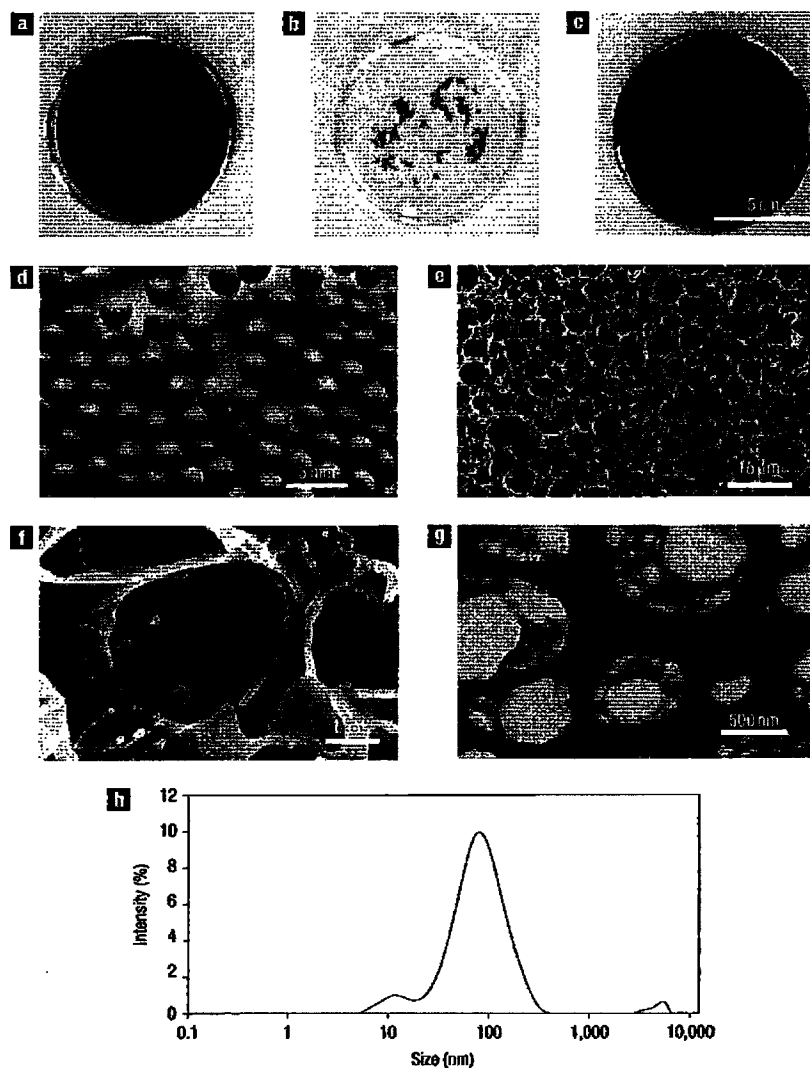


Figure 1 Characterization of solid nanocomposites and nanodispersions. **a–c**, Visual comparison of an aqueous nanodispersion of Oil-Red (OR) (**a**), OR powder added to water (**b**), and OR dissolved in acetone (**c**). **d**, Highly porous nanocomposite beads (poly(vinyl alcohol)/sodium dodecyl sulphate) containing OR (0.018 wt%). **e**, SEM image of the internal porous bead structure. **f**, Higher magnification showing nanoparticles associated with the edge of a pore. **g**, STEM image of OR nanoparticles after drying the nanodispersion onto a porous carbon grid. **h**, Dynamic light scattering analysis of the OR nanodispersion showing an average particle size of 90 nm.

loadings up to 0.5 wt%. SEM imaging (see Supplementary Information, Fig. S2) showed significantly more OR particles in the resulting composite. Acetone destabilized the emulsions somewhat, but rapid freezing still allowed porous emulsion-templated structures. After the addition of water, STEM observations and DLS measurements (see Supplementary Information, Figs S3 and S4) showed significantly larger average particle diameters (~ 300 nm).

OR can be solubilized in water by conventional PVA/SDS micellization. We therefore evaluated the potential of our system to disperse excess organic material to a greater extent than simple solubilization over various PVA/SDS ratios. All combinations were above their respective critical micelle concentrations (see Supplementary Information). The formation of OR-saturated micelles (SM) in water (300 h at 20 °C) was studied for each PVA/SDS ratio (Fig. 2a). These values form a baseline

solubilization plot (Fig. 2b), representing the maximum OR concentration achievable at each ratio. For comparison, a series of emulsion-templated samples was produced with comparable SDS/PVA ratios and dispersed into water to produce OR nanodispersions (Fig. 2c). Nanodispersions with high stability (<14 days) (see Supplementary Information, Fig. S5) required both SDS and PVA (Fig. 2c). A sixfold increase in OR concentration (6.5×10^{-3} M OR, PVA/SDS = 7×10^{-3} M/0.3 M) was achieved over the saturated micelle concentration (SMC). Moreover, this was achieved only seconds after the addition of water, compared to extended SM equilibration times (>300 h). As the nanodispersions are formed almost instantly, they may be deployed at the point of use without requiring high-temperature, high-shear mixing or relying on molecular solubility. Higher loadings (more than 50 \times greater than the SMC) were achieved by further increasing the OR concentration in the oil phase;

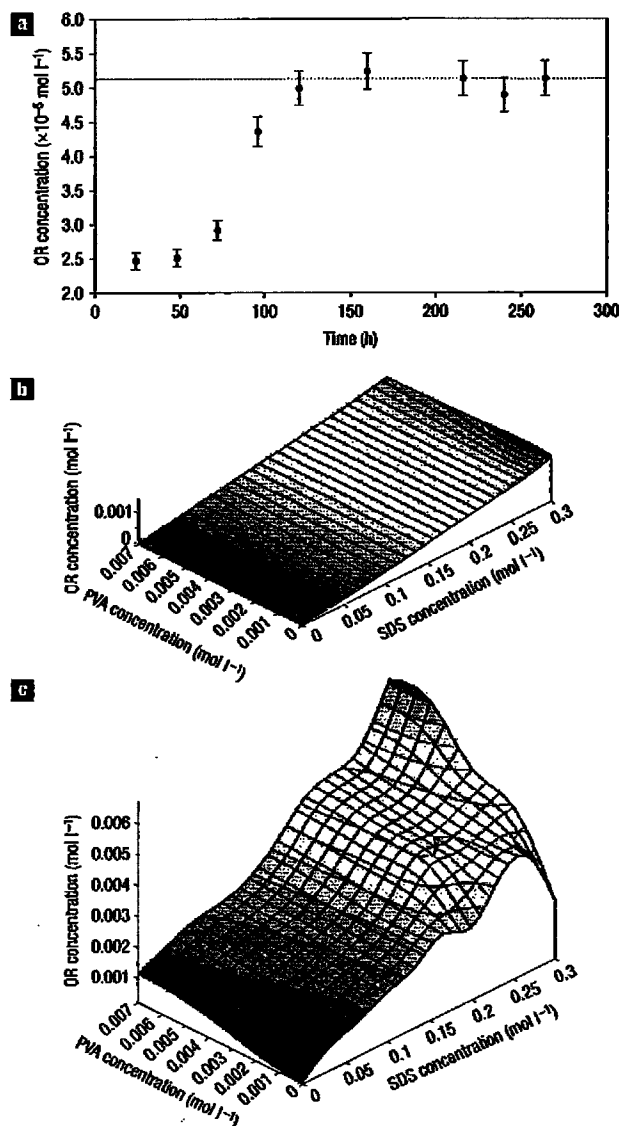


Figure 2 Comparison of conventional surfactant solubilization and nanodispersions of Oil-Red (OR). **a**, Saturation kinetics of OR in an aqueous micellar solution of SDS ($\text{SDS} = 0.01 \text{ mol l}^{-1}$; $\text{PVA} = 0$). The horizontal line indicates equilibration. **b**, Baseline solubilization of OR using various SDS and PVA ratios. The plot represents maximum OR solubilization within given polymer and surfactant concentrations. **c**, Aqueous concentration of OR achieved using emulsion-templated nanodispersion technology (identical ratios of PVA and SDS to **b**). The difference between **b** and **c**—the 'excess' concentration over the saturated micelles—indicates the presence of OR nanoparticles within the 'solution-like' dispersion.

however, larger OR particles ($>300 \text{ nm}$) with reduced stability (2 days) were formed. Zeta-potential measurement (see Supplementary Information, Fig. S11) showed negatively charged nanoparticles, indicating SDS surface adsorption (low OR loading = $-23.3 \pm 1.2 \text{ mV}$; high OR loading = $-21.8 \pm 0.188 \text{ mV}$).

The Horn and Rieger¹ categories have several common features. Apart from precipitation, the methods use mobile/liquid emulsions containing water-insoluble compounds dissolved within the oil phase. As the oil is slowly, controllably and selectively removed,

the oil droplets shrink, and the solute concentrates and ultimately solidifies. Our technique solidifies the entire emulsion by freezing to form static oil droplets. Whole-droplet motion is prevented during the simultaneous sublimation of both frozen water and solvent. In contrast to other techniques, the oil droplets are depleted of oil in the presence of a static surface structure or support.

The mechanism of nanoparticle formation is complex; however, the processes operating during conventional freeze-drying of aqueous solutions are well understood^{1,15–17}. Initial freezing generates ice crystals of pure water¹⁸, leading to dramatic concentration and viscosity increases within the remaining liquid¹. The resulting desiccation is highly efficient ($>99\%$ water removal from the solute) and causes phase separation¹⁵. Crystallization of the concentrated liquid regions is viscosity-inhibited¹⁷, and final solidification produces crystalline, amorphous or mixed solids. Supercooling is at its highest when liquid nitrogen is used to freeze small volumes¹⁵, leading to significant liquid stresses¹⁶. Our technique involves the freezing/freeze-drying of emulsions (volumes ranging from $\sim 6 \times 10^{-8} \text{ cm}^3$ for atomization to $\sim 2 \text{ cm}^3$ for direct freezing) and similar concentration of the solute in the organic phase occurs¹⁸. The individual, isolated oil droplets have very small volumes ($\sim 5 \times 10^{-10} \text{ cm}^3$ for beads) and undergo significant local supercooling and concentration during freezing (Fig. 3b). Areas of saturated solute will develop that are subject to precipitation/crystallization during water/oil removal (Fig. 3c). Particles thus formed will be dispersed upon addition of water and dissolution of the solid porous polymer/surfactant support. PVA is a known dispersion stabilizer, binding extremely strongly to nanoparticle surfaces¹ and contributing to dispersion stability (Fig. 3d).

The formation of dry, redispersible solids containing nanoparticles offers significant advantages over liquid-dispersion storage and transport. OR/PVA beads formed identical clear, red nanodispersions after two years without stringent storage conditions. As a result of using rapid freezing, emulsion stability is not critical and the choice of surfactants, hydrophilic polymers, solvents and organic active ingredients is therefore very broad, including biodegradable polymers and temperature-sensitive drugs. Nanoparticle loadings were adjusted by changing the O/W ratio with available oil-phase fractions from 0.01 to at least 0.9. Porosity (Fig. 4a–c), dissolution kinetics and material properties are also tuneable by means of this mechanism, in contrast to the reported spray-freezing-into-liquid processes for organic microparticle preparation^{19,20}.

We have produced porous surfactant/silica powders (Fig. 4e–f) with our process, avoiding spray pyrolysis or templating/etching routes^{21–23}. Also, to remove the reliance on volatile organic solvents, we have recently adopted liquid CO_2 (refs 24–28) as the emulsion oil phase. After directly freeze-drying the CO_2 /water emulsions, porous polymer monoliths that dissolve rapidly in water and form clear OR nanodispersions (diameter $\sim 240 \text{ nm}$) were formed (see Supplementary Information, Fig. S9).

The practical value of the approach was demonstrated by forming aqueous nanodispersions of poorly soluble commercial organic biocides, which are typically used in products ranging from cosmetics to agrochemicals. For example, Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) has low water-solubility ($10 \mu\text{g ml}^{-1}$) and limited biocidal activity without the use of high doses, co-solvents or oil phases²⁹. Also, micellization is known to decrease bactericidal activity²⁹. The minimum inhibitory concentration (MIC_{50}) against the Gram-negative bacterium *Corynebacterium* of an ethanol/water solution of Triclosan/PVA/SDS was compared with the MIC_{50} of aqueous nanodispersions prepared by our route.

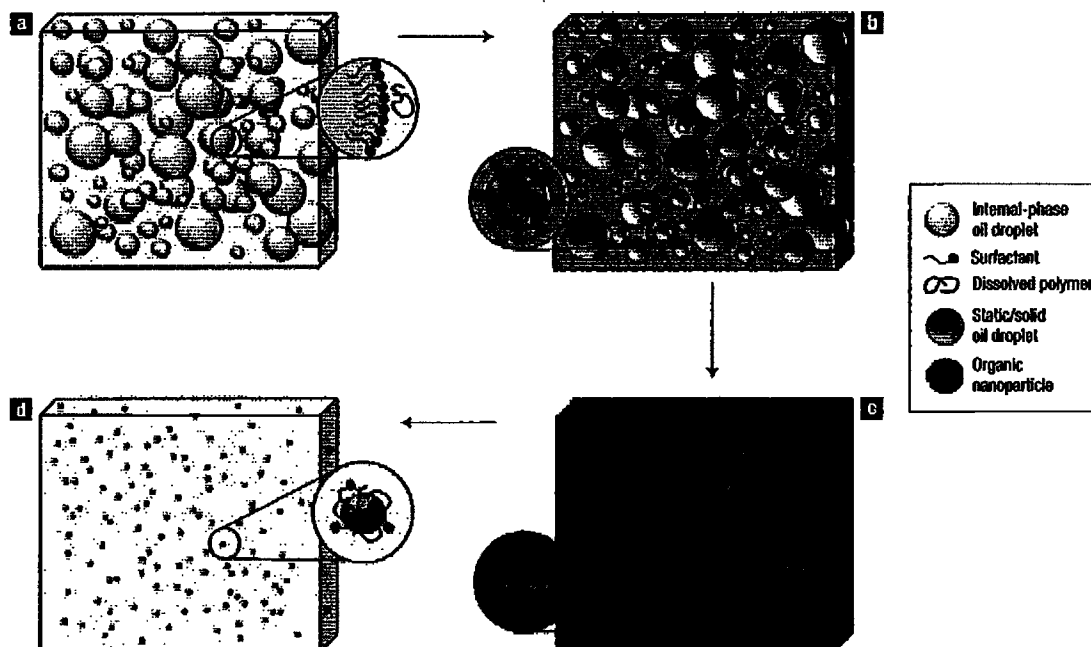


Figure 3 Schematic/mechanistic representation of nanoparticle formation during freeze-drying. **a**, Oil-in-water emulsion; organic compound dissolved in internal-phase oil droplets. Enlarged area: homogeneous, surfactant-stabilized oil droplet and dissolved polymer. **b**, Frozen oil-in-water emulsion; static/solid oil droplets within a solidified continuous phase. Enlarged area: organic compound inhomogeneously distributed within the frozen oil droplet and associated surfactant. **c**, Emulsion-templated porous solid after freeze-drying. Enlarged area: organic nanoparticles within open pores. **d**, Organic nanodispersion after addition of water. Enlarged area: surfactant- and polymer-stabilized organic nanoparticle.

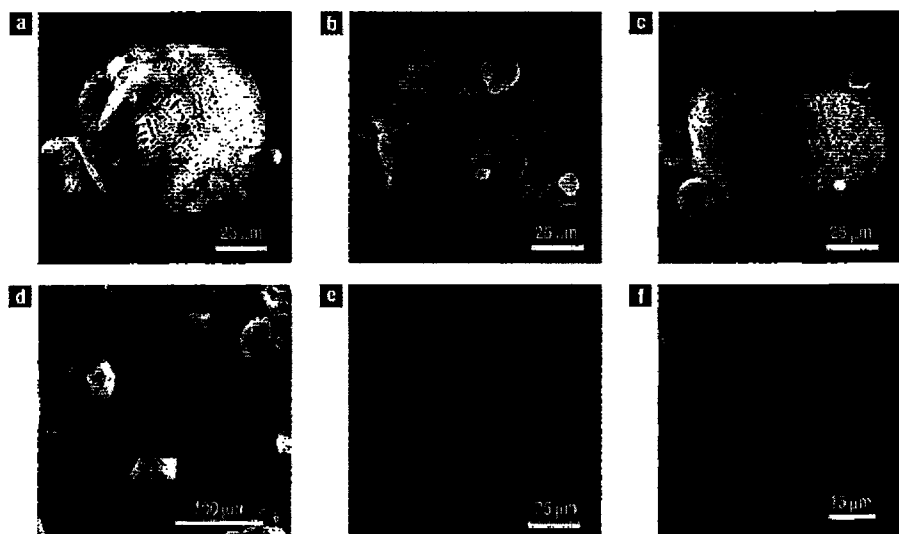


Figure 4 Scanning electron microscopy images of freeze-dried atomized powders. **a–c**, Freeze-dried PVA/SDS powders with 0% emulsion-templating (**a**), 20% emulsion-templating (**b**), and 50% emulsion-templating (**c**). **d**, Low-magnification image of powder with 50% emulsion-templating. **e**, Emulsion-templated powder containing SDS only (66.7% emulsion-templating). **f**, Emulsion-templated silica powder after calcination; original emulsion contained PVA and silica HS-30 (75% emulsion-templating).

The ethanol/water system showed substantial inhibition of regrowth of the bacterium at Triclosan concentrations >100 p.p.m. (Fig. 5a). Inhibition was lost rapidly at concentrations <100 p.p.m., with an observed MIC_{50} of approximately 50 p.p.m. In contrast,

aqueous nanodispersions were significantly more active (2.5% regrowth at 50 p.p.m.), with bacterial regrowth inhibited to $<50\%$ at 6.25 p.p.m. (Fig. 5b). In both cases, 100% regrowth of *Corynebacterium* was observed between 1.56 and 0.78 p.p.m.,

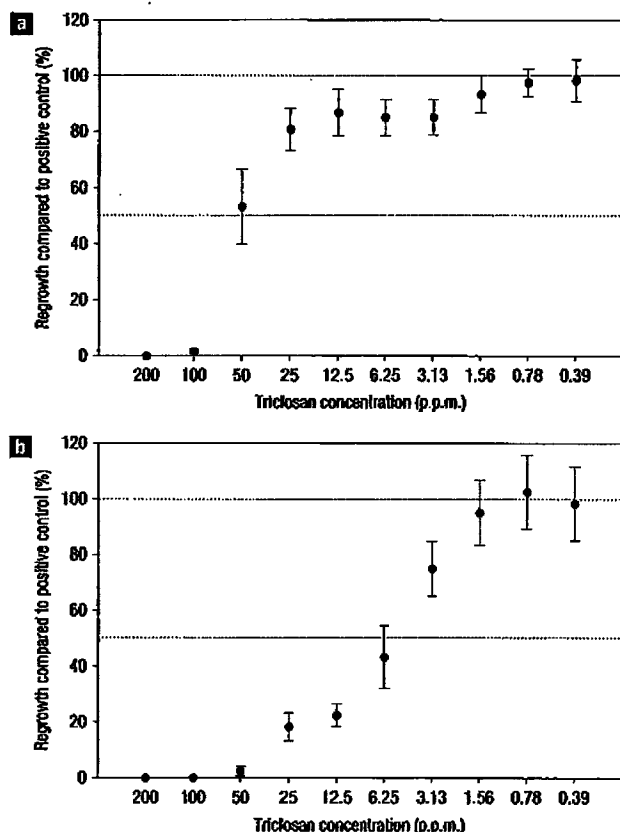


Figure 5 Evaluation of the minimum inhibitory concentration (MIC) of different forms of the antibacterial agent Triclosan. a,b, Regrowth profiles of the Gram-positive bacterium *Corynebacterium* in the presence of an aqueous/ethanol solution of Triclosan/SDS/PVA (a), and aqueous Triclosan nanodispersion with SDS and PVA present (b). The horizontal lines depict the MIC₅₀ (lower) and complete regrowth (upper) relative to positive control. Error bars are derived from ten repeat measurements. All measurements were conducted in a brain/heart infusion.

suggesting similar biocidal mechanisms at low concentrations and possibly indicating nanoparticle dissolution at these concentrations. The observed MIC₅₀ reduction (one-eighth of the solution MIC₅₀) without the use of co-solvents is of clear interest across many applications and our ongoing experiments are focused on establishing the mode of action.

To summarize, we have demonstrated the generic formation of organic nanodispersions by freeze-drying emulsions containing water-insoluble compounds dissolved in a volatile oil phase. Water-soluble materials present in the aqueous phase form a porous solid support that dissolves rapidly on addition of water to disperse the nanoparticles. This technique has wide applicability, because nanodispersions are a valuable alternative to molecular solutions. Water-insoluble commercial biocides showed increased activity, suggesting the potential for decreased application doses and lower rates of acquired bacterial resistance.

METHODS

VARIATION OF POROSITY BY MEANS OF INTERNAL-PHASE VOLUME CONTROL

A range of 'unloaded' porous PVA/SDS materials were produced using oil-phase fractions of 0, 0.2, 0.4, 0.6 and 0.75 (see Supplementary Information,

Fig. S6) to demonstrate the control of porosity using our technique. Materials produced with no oil phase present showed porosity derived solely from ice-templated macropores, formed by phase separation during the freezing process¹⁸. As the O/W ratio increases, the number of emulsion-templated pores increases accordingly. At an internal-phase fraction of ~0.75, the porosity is derived solely from the emulsion-templating process, as supported by pore volume measurements (see Supplementary Information, Fig. S7). Materials with larger pore volumes were found to dissolve more rapidly than those with lower pore volumes and hence the dissolution kinetics can be fine-tuned (see Supplementary Information, Figs S7 and S8).

CO₂ METHODS

CO₂-in-water emulsions, containing OR dissolved in the liquid CO₂ phase and PVA in the aqueous phase, were frozen by liquid nitrogen immersion followed by freeze-drying (see Supplementary Information). The emulsions were stabilized by an inexpensive triblock copolymer, poly(vinyl acetate)-*b*-poly(ethylene glycol)-*b*-poly(vinyl acetate), which was synthesized as reported earlier²⁸.

MIC₅₀ EVALUATION

Biocidal activity was measured against a Gram-negative bacterium, *Corynebacterium*, in a standard growth medium, and regrowth was compared against positive controls. Triclosan was either dissolved into an aqueous brain/heart infusion (BHI) containing 30% ethanol or dispersed into BHI with no additional solvent to form initial concentrations of 400 p.p.m. (PVA and SDS were also present in the BHI/ethanol comparison to match the nanocomposite composition: Triclosan/SDS/PVA = 10/63/27 wt%). Subsequent dilution with either BHI/ethanol or BHI alone generated a series of lower concentrations, which were inoculated with *Corynebacterium*. After plating into wells, the samples were incubated at 37 °C for 18 h. All measurements were repeated ten times to ensure significance. MIC₅₀ values were assessed as the concentration required to maintain <50% bacterial regrowth relative to the control. All control experiments included SDS and PVA and additional ethanol as required.

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Author contributions

All authors contributed to the experimental programme, either through design of the experiment or practice. D.T. led the microbiological testing. H.Z., A.C. and S.R. co-wrote the manuscript.

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ATTACHMENT 5

Emulsion Templating

PVA – polymer in aqueous phase

